

A LABORATORY HANDBOOK
FOR THE
ANALYSIS
OF
MILK, BUTTER AND CHEESE.

EVANS.

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A LABORATORY HANDBOOK

FOR THE

ANALYSIS

OF

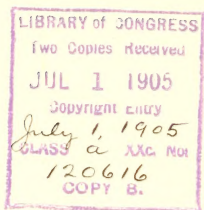
MILK, BUTTER AND CHEESE,

BY



JAMES RITTENHOUSE EVANS, B. S.

Member of The American Chemical Society, The Society of Chemical Industry, The American Waterworks Association, Etc.



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PREFACE TO SECOND EDITION.

Since the publication of the first edition, the author has had an opportunity to test the following pages on a class of students absolutely unfamiliar with Milk and Butter analysis. The results were exceedingly gratifying, in that while access was obtainable to all the more complete works on the subject, the following notes seemed to afford the greatest help in actual laboratory work.

As stated in the preface to the first edition, no attempt has been made to interpret the results, the object being to eliminate everything not absolutely necessary to the complete understanding of the actual work of a determination. By following this system, it is hoped that much of the lack of clearness caused by an exhaustive treatment of the subject has been overcome.

The text itself has been carefully corrected and revised, and it is hoped that the present edition will contain few, if any, mistakes.

During the past year, a rigid investigation was undertaken for the purpose of determining the comparative value of the various "milk testers," with the result that the lactoscope, creamometer, pioscope, lactobutyrometer and the lactometer, when used alone, were found absolutely worthless in the testing of a milk. The investigation has shown, however, that real merit exists in the centrifugal machines of the Babcock type, when carefully and intelligently used. As a result, an appendix has been added, describing the Babcock tester and its method of use.

Several tables have also been added, and additional instructions given wherever it has been found necessary or advantageous.

J. R. E.

PREFACE TO FIRST EDITION.

This little handbook is intended merely as a laboratory guide to the chemist who has to deal with the chemical analysis of milk and its products. No attempt has been made to discuss the results, beyond giving, in the form of an Appendix, a copy of the definitions and standards decided upon by the Department of Agriculture.

The methods given are well known, the only attempt at originality being in the way they are arranged in distinct steps, this form of arrangement being found, by the author, to be to some extent, an aid to clearness.

No mention has been made of the various methods of testing milk by the aid of instruments such as the lactoscope, creamometer, etc., their unreliability being well known.

J. R. E.

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Analysis of Milk.

ANALYSIS OF MILK.

DETERMINATION OF SPECIFIC GRAVITY.

a—By the Hydrometer.

1. Fill the Hydrometer tube with the carefully mixed milk, avoiding air-bubbles, to within four inches of the top.
2. Insert the Hydrometer (preferably that used by the N. Y. B. H.), taking care that the graduated tube is not wet above the mark to which it sinks, and that the Hydrometer does not touch the sides of the jar.
3. Read the top of the merniscus.

N. B.—The N. Y. B. H. hydrometer or lactometer reads in degrees, which must be converted by means of the following table to its corresponding value in terms of the specific gravity of water.

TABLE SHOWING SPECIFIC GRAVITIES CORRESPONDING TO
DEGREES OF THE NEW YORK BOARD OF
HEALTH LACTOMETER.
Temperature, 60° F.

DEGREE.	SP. GR.	DEGREE.	SP. GR.
90	1.02619	106	1.03074
91	1.02639	107	1.03103
92	1.02668	108	1.03132
93	1.02697	109 ..	1.03161
94	1.02726	110	1.03190
95	1.02755	111	1.03219
96	1.02784	112	1.03248
97	1.02813	113	1.03277
98	1.02842	114	1.03306
99	1.02871	115	1.03335
100	1.02900	116	1.03364
101	1.02929	117	1.03393
102	1.02958	118	1.03422
103	1.02987	119	1.03451
104	1.03016	120	1.03480
105	1.03045		

b—By the Specific Gravity bottle.

1. Fill the bottle to the mark with recently distilled water.
2. Weigh exactly, taking care that the bottle is perfectly dry on the outside.
3. Pour out the water and dry thoroughly.
4. Fill with milk to the mark, avoiding frothing as much as

possible, and dislodging any air bubbles clinging to the sides of the bottle.

5. Weigh accurately.

6. The weight of the water and flask, divided into the weight of the milk and flask, is equal to the specific gravity of the milk, at the temperature at which the operation is performed.

c—By the Westphal Balance.

1. Weigh in air a piece of glass, or metal, suspended from the arm of the balance by means of a fine copper wire.

2. Weigh the same in distilled and recently boiled water. The water should be contained in a beaker placed on the floor of the balance.

3. Dry the piece of glass, or metal, thoroughly and weigh in milk, avoiding air-bubbles and frothing.

Then :

Let P = Weight in air.

P_1 = Weight in water.

P_2 = Weight in milk.

D = Specific gravity of milk.

$$D = \frac{P - P_2}{P - P_1}$$

NOTE—The specific gravity, as obtained at any certain temperature, should be corrected to that at 15.5° Cent., or 60° Fahr. This may be done by using Vieth's table, given in Blyth's "Foods: their Composition and Analysis," fifth edition, page 216.

DETERMINATION OF WATER, TOTAL SOLIDS AND ASH.

1. Weigh out about 3 grams of milk into a flat platinum dish, previously weighed, not less than 5 to 10 cm. in diameter.

2. Evaporate to dryness on water bath.

3. Heat in water oven to constant weight. This will take about three or four hours.

4. Cool in dessicator and weigh. The weight of residue is equal to the weight of TOTAL SOLIDS in the weight of milk taken. The difference between this weight and the weight taken, is the weight of WATER. Calculate both weights to percentages.

5. Gently ignite residue at low redness, until ash is white, or nearly so, breaking up all lumps with a platinum wire.

6. Cool in dessicator and weigh.

7. Weight of final residue is equal to the weight of ASH, in the weight of milk taken. Calculate to percentage.

DETERMINATION OF FAT.

Adams' Method.

1. Roll up a strip of fat-free blotting paper into a cylinder, fasten it with a piece of fine wire and place in a weighing tube. Dry at 105° Cent., for one hour.

2. Cool and weigh.

3. Pour on the paper, from a pipette, holding the paper upright and pouring on from the top, about 5 c.c. of milk.

N. B.—It is better to apply the milk to the inside of the cylinder.

4. Replace the cylinder quickly in weighing-tube and weigh.

5. The increase in weight is equal to the weight of the milk taken.

6. Place cylinder in water oven at 100° Cent., for one or two hours.

7. Place in Soxhlet extraction apparatus and extract for two and one-half hours with anhydrous Ether, or refined Gasoline.

8. Remove the cylinder from the extractor and vaporize the

Ether, or Gasoline, from the paper by aid of suction, or gentle heat.

9. Place in weighing-tube, when perfectly dry, and weigh. The weight thus obtained, minus the weight of cylinder and tube, obtained at (2) will give the weight of the SOLIDS NOT FAT. This weight subtracted from the weight of the Total Solids will give the weight of FAT.

The weight of FAT may be determined directly, if, before beginning the extraction, the flask of the extraction apparatus is weighed in a perfectly dry condition, and after the extraction of the fat the Ether extract is evaporated to dryness in it, the residue dried in the water oven to constant weight and the flask and contents again weighed. The increase in weight is equal to the weight in FAT. Do not keep in water oven too long, as the fat has a tendency to increase in weight.

ESTIMATION OF LACTOSE BY DIFFERENCE.

Blyth.

1. Proceed as if the Total Solids were to be found and extract residue with Ether.
2. Extract the dry fat-free residue with weak, boiling, Alcohol.
3. Filter and evaporate the Alcohol extract to dryness in a platinum dish.
4. Heat in water oven to constant weight.
5. Cool in dessicator and weigh.
6. Ignite residue until all Carbon has been burned off and ash is white, or nearly so.
7. Cool in a dessicator and weigh.
8. The difference between these two weighings is the weight of the Lactose in the weight of milk taken. Calculate to percentage.

POLARIMETRIC METHOD OF ESTIMATING LACTOSE IN MILK.

Method of Dr. W. Wiley.

1. Take 60 c.c. of milk.
2. Add 10 c.c. of a solution of Mercuric Nitrate (made by dissolving Mercury in twice its weight of Nitric acid (sp. gr. 1.42) and diluting with four volumes of water) and dilute mixture to 100 c.c.
3. Shake and filter through a dry filter.
4. Observe rotation at once with polariscope.
5. Take the specific rotation of milk sugar as $(\alpha)_d = 52.5$ degrees. Calculate the weight of anhydrous sugar by following formula :

$$S = \frac{100 \times d}{52.5 \times l} \times \frac{100 - (1.075 F \times .8P)}{Q} \times \frac{.95}{\text{Sp. Gr.}}$$

Where Q = Quantity of milk taken.

F = Weight of fat in quantity Q.

P = Weight of proteids in quantity Q.

l = Length of tube.

d = Angular rotation found.

DETERMINATION OF TOTAL PROTEIDS.

Kjeldahl's Method.

1. Weigh out about 5 grams of milk into a Kjeldahl flask fitted with a balloon stopper.
2. Add 20 c.c. strong Sulphuric acid.
3. Add a small globule of Mercury.
4. Place flask, stoppered, in an inclined position and heat nearly to the boiling point.
5. Keep at this temperature for 15 minutes.
6. Increase heat until liquid boils freely.

7. Boil until liquid becomes clear, or at most, of a pale straw color. Remove from the flame, keeping in an upright position.

8. Add at once, crystals of Potassium Permanganate until liquid remains of a purple or green color after shaking.

9. Transfer liquid to a liter distillation flask containing about 200 c.c. of ammonia-free water, a few pieces of granulated Zinc and 25 c.c. of Potassium Sulphide solution (40 grams to the liter).

10. Shake until all are thoroughly mixed.

11. Add 50 c.c. of a saturated solution of Sodium Hydrate in ammonia-free water.

12. Connect flask with a 600 m.m. Liebig Condenser, the end of which dips into a solution of accurately standardized decinormal Sulphuric Acid.

13. Distil at a moderate heat until all the ammonia has gone over.

14. Titrate the acid against a carefully standardized alkali solution, using Cochineal as an indicator, and determine the amount of standard acid used up by the distillate. Convert corresponding amount of ammonia to Nitrogen and multiply this result by 6.25 to convert to milk proteids.

N. B.—Care must be used in this determination to prevent the breaking of the flasks. The distilling apparatus must be freed from ammonia by adding C. P. water to the reagents and distilling until clean, previous to the actual determination.

DETERMINATION OF CASEIN AND ALBUMEN.

1. Take about 100 c.c. of milk and weigh accurately.

2. Divide into three approximately equal portions.

3. Dilute the first portion 4 times and acidify with Acetic acid until the Casein coagulates.

4. Pass Carbon Di-oxide through the liquid and let stand until precipitate settles.

5. Siphon off the whey into the second portion and, if necessary, add more acid.
6. Pass more Carbon Di-oxide and again allow to settle.
7. Siphon on to the third portion and repeat the same treatment with it.
8. Collect all the Casein on a weighed filter.
9. Wash dry and weigh. Weight equals CASEIN.
10. Take the weigh and filtrate in a large beaker and raise to the boiling point. Boil ten minutes.
11. Collect the Albumen on a weighed filter.
12. Wash, dry in water oven and weigh. Weight equals ALBUMEN.

ESTIMATION OF ACIDITY.

1. Take 100 c.c. of milk.
2. Titrate in beaker with $\frac{N}{10}$ NaOH solution, using Phenolphthalein as an indicator.
3. Call each cubic centimeter of alkali used one degree, and report in degrees of acidity.

DETERMINATION OF LACTIC ACID IN MILK.

1. Take 5 grams, approximately, of milk. Weigh accurately.
2. Evaporate to dryness in platinum dish on water bath.
3. Extract fat with Carbon Di-sulphide.
4. Treat residue with an Alcoholic solution of Oxalic acid.
5. Filter and wash.
6. Add an excess of Hydrated Oxide of lead to filtrate.
7. Filter and wash.
8. Saturate filtrate with Hydrogen Sulphide.
9. Filter and wash.
10. Concentrate by evaporation.
11. Add Zinc Oxide and boil.

12. Filter and wash.
13. Evaporate filtrate to a small bulk,
14. Let stand for some time.
15. Filter off the crystals of Lactate of Zinc on a weighed filter.
16. Wash with Alcohol and weigh.

Calculate the Lactic Acid. The composition of the Zinc Salt is $2 (C_3H_5O_3) Zn + 3H_2O$.

ANALYSIS OF CREAM AND CONDENSED MILK.

ANALYSIS OF CREAM.

As Cream contains the same constituents, exactly, as Milk, differing from the latter only in the proportion of the constituents to each other, its analysis clearly follows the same lines. The following points should, however, be noticed :

- 1st. Smaller quantities should in all cases be taken.
- 2d. The fat should be extracted for a longer period of time, say, three and one-half hours instead of two and one-half.
- 3d. If desired, the cream may be diluted a certain definite amount and the diluted solution used for the analysis, correcting the results, of course, to correspond with the degree of dilution.
- 4th. The same adulterations are to be looked for as in milk.

ANALYSIS OF CONDENSED MILK.

The sample is to be thoroughly mixed and accurately weighed, about 15 grams being taken, and then diluted with water so as to make a 10% solution, all results being carefully corrected in accordance with the dilution.

TOTAL SOLIDS—Use 10 c. c. of 10% solution.

ASH—Ditto.

FAT—Use for extraction, Petroleum Spirit, or a mixture of this with anhydrous Ether, (15% of Ether).

TOTAL PROTEIDS—10 c.c. of a 50% solution of milk are diluted with water, and 5 c.c. of a 6% solution of Sulphate of Copper added. It is then almost neutralized with deci-normal NaOH solution and the precipitate filtered off on a weighed filter, washed, and the Nitrogen in filter and contents determined as in milk. The Nitrogen contained in a similar filter should be determined and subtracted. (Ritthausen's Process).

LACTOSE—As in milk.

CANE SUGAR—An approximation may be made by deducting the proteids, ash, fat and milk sugar from the total solids. For a more exact method, see Blyth's "Foods: their Composition and Analysis," fifth edition, page 264.

Blyth gives the following formulas for calculating the original composition of the milk and the degree of concentration.

	$\text{Fat} \times 3.4$
Fat of original Milk	Proteids of Condensed Milk.
	Ash of Cond. Milk $\times 8.9$
Ash of original Milk	Non Fatty Solids of Cond. Milk.
	Total Milk Solids of Cond. Milk
Degree of Concentration	Total Milk Solids of Orig. Milk.

DETECTION OF ADULTERATIONS IN MILK.

THE ADDITION OF WATER.

To detect the addition of water to milk, it is necessary to know the percentage of Solids and Fat. The Dept. of Agriculture, in this country, (See Appendix I) requires that the percentage of Fat in Whole Milk (obtained by the complete milking of one or more healthy cows) shall not fall below 3.25% nor the percentage of Solids not Fat, in such milk, below 8.5%, nor the Total Solids below 12%.

The probable amount of added water can be obtained by the following formula given by Blyth:

$$\text{Percentage of Added Water} \times 100 = \frac{S \times 100}{8.5}$$

S = Solids not Fat in Sample.

THE ABSTRACTION OF FAT.

This adulteration may be detected by observing the ratio of the percentage of Solids not Fat to the percentage of Fat, or by the falling of the percentage of Fat below the standard of 3.25%. The ratio between the two should not be far from the ratio of 9 to 4:

The probable percentage of abstracted Fat may be found by the following formula given by Blyth:

$$\text{Percentage of Fat abstracted} = 100 \frac{3.25 - f}{3.25}$$

f = Percentage of Milk Fat in Sample.

THE ADDITION OF WATER AND ABSTRACTION OF FAT.

This adulteration is practiced in order to bring the specific gravity to a normal reading and make the physical testing instruments read the same as for a good milk. It may be detected as above.

THE ADDITION OF SEPARATED OR SKIM MILK.

This is probably the most common form of adulteration and can only be detected by the falling of the percentage of the Fat below the standard. Of course, if the original composition of the milk is known, it is not hard to detect this fraud.

THE ADDITION OF PASTEURIZED OR STERILIZED MILK.

This is not a common form of adulteration. Faber claims that it may be detected by the small amount of soluble Albumen. Blyth gives the following test for a milk which has been heated :

1. Add to 5 c.c. of milk $\frac{1}{2}$ gram of Paraphenylene-diamine.
2. Add 2 drops of a 10% solution of Hydrogen Peroxide.
3. Prepare at the same time another tube of good fresh milk to use as a check. Compare colors and draw conclusions.

Fresh Milk.....Blue.

Pasteurized Milk.....Faint Blue.

Sterilized Milk.....Colorless.

N. B.—The amount of soluble Albumen in normal fresh milk runs between .41 per cent, and .45 per cent.

THE ADDITION OF STARCH.

1. To 100 c.c. of sample add a solution of Iodine in a solution of Potassium Iodine. (A few drops are sufficient).
2. A blue color indicates Starch.

THE ADDITION OF SUGARS OTHER THAN LACTOSE.

Cotton's Method.

1. Take 10 c.c. of milk.
 2. Add 5 grams of powdered Ammonium Molybdate.
 3. Add 10 c.c. dilute Hydrochloric acid (1 — 10).
 4. Make up tube of good fresh milk in same way.
 5. Compare colors after heating both tubes at 80° Cent., for a few moments. The tube containing sugar will become blue.
-

DETECTION OF THE ADDITION OF PRESERVATIVES TO MILK.

THE ADDITION OF BORAX AND BORAIC ACID.

Detection.

1. Evaporate 10 c.c. of milk, previously made alkaline with Sodium Hydrate to dryness.
2. Char the residue.
3. Add a little water and boil.
4. Acidify with HCl.
5. Dip into the liquid a piece of Tumeric paper.
6. Dry over a flame, being careful not to char paper.
7. Moisten with Ammonium Hydrate.
8. A dark blue coloration indicates a borate.

N. B.—The dark red coloration nearly always produced must not be taken for the test.

Estimation.*R. T. Thompson's Method.*

1. Take 100 c.c. of milk and make distinctly alkaline with Sodium Hydrate.
2. Evaporate to dryness in a platinum dish.
3. Char thoroughly.
4. Add 20 c.c. of water.
5. Heat.
6. Add Hydrochloric acid, drop by drop, until only Carbon remains.
7. Transfer to a 100 c.c. flask, washing out dish with water into flask.
8. Add 0.5 gram dry Calcium Chloride.
9. Add a few drops of Phenolphthalein solution.
10. Run in a 10 % solution of Sodium Hydrate until a permanent pink color is obtained.
11. Add 25 c.c. saturated lime water.
12. Make up to exactly 100 c.c. with water.
13. Shake well.
14. Filter through a dry filter into a 50 c.c. flask.
15. When filtrate has just reached the mark, remove from under funnel.
16. Add normal Sulphuric acid until the pink color disappears.
17. Add a few drops of a solution of Methyl Orange.
18. Then add the acid until the yellow color changes to pink.
19. Add very carefully $\frac{N}{5}$ NaOH until the yellow just appears.
20. Boil.
21. Cool.
22. Add 30 % of the volume of Glycerine. That is, 30 % of the resulting mixture.
23. Titrate with $\frac{N}{5}$ NaOH until a permanent pink color is obtained.

24. Read burette. Each cubic centimeter of the $\frac{N}{5}$ NaOH solution used is equal to .0124 Boric acid or .007 B_2O_3 . Multiply weight thus found by two and calculate percentage.

THE ADDITION OF FORMALIN.

Heyner's Method.

1. Take 10 c.c. of milk in a test tube.
2. Add one drop of a solution of Ferric Chloride.
3. Dilute to 30 c.c.
4. Pour concentrated Sulphuric acid down the side of the tube so as to form a layer at the bottom.
5. A violet ring at the junction of the two liquids indicates Formic Aldehyde.

THE ADDITION OF GELATINE.

Allen's Method.

1. Take 10 c.c. of the milk in a test tube.
2. Add 10 c.c. of the acid Mercuric Nitrate solution.
3. Shake.
4. Add 20 c.c. of water.
5. Shake again and let stand 5 minutes.
6. Filter. In presence of much Gelatine the filtrate will be cloudy.
7. Take 10 cc. of the filtrate and add 10 c.c. of an aqueous solution of Picric Acid.
8. A yellow precipitate will occur if much Gelatine be present. Smaller amounts can be detected by the cloudiness of the solution.

N. B.—The acid Mercuric Nitrate is prepared by dissolving Mercury in twice its weight of Nitric Acid of 1.42 Sp. Gr. and diluting this solution to 25 times its bulk with distilled water.

THE ADDITION OF BENZOIC ACID.

Blyth.

1. Take 200 c.c. of milk.
2. Make alkaline with Barium Hydrate.
3. Concentrate to 50 c.c.
4. Add Calcium Sulphate, mix to a paste, and dry on water bath.
5. Powder, and moisten with dilute Sulphuric acid.
6. Extract with 50 % Alcohol.
7. Neutralize the Alcohol extract with Barium Hydrate.
8. Evaporate to a small bulk.
9. Acidulate with Sulphuric acid.
10. Extract with Ether.
11. Evaporate Ether in a flask, by aid of suction.
12. Dissolve residue from Ether, in water.
13. Add a little Sodium Acetate.
14. Test for Benzoic acid with a solution of Ferric Chloride.

NOTE.—If the acid is to be determined, sublime the residue and weigh the sublimate thus obtained, checking the result with the loss of weight the residue has undergone.

THE ADDITION OF SALICYLIC ACID.

1. Acidify 100 c.c. of milk with Acetic acid.
2. Heat.
3. Filter off whey.
4. Add Hydrochloric acid.
5. Shake up whey with Ether in a separatory funnel.
6. Separate the Ether extract.
7. Evaporate it to dryness, by aid of suction.
8. Dissolve residue in water.
9. Add a neutral solution of Ferric Chloride.
10. A violet coloration indicates Salicylic acid.

THE ADDITION OF SODIUM CARBONATE.

1. Evaporate 10 c.c. of milk to dryness in a platinum dish.
2. Ignite until dish is white or nearly so.
3. Add two drops of Hydrochloric acid.
4. An effervescence indicates a carbonate.

THE ADDITION OF B-NAPHTHOL COMPOUNDS.

Leffman and Beam.

1. Take 200 c.c. of milk in a distillation flask.
2. Acidify with Sulphuric Acid.
3. Connect flask with a Liebig condenser.
4. Distil until 150 c.c. distillate have been produced.
5. Shake distillate up with 20 c.c. of Chloroform.
6. Separate Chloroform extract in a separatory funnel.
7. Make alkaline with Potassium Hydrate.
8. Heat nearly to boiling.

Color changes take place as follows :

Salol Light red.

Phenol Light red—to brown—to colorless.

B-naphthol Deep blue—to green—to brown.

DETECTION OF THE ADDITION OF COLORING MATTERS IN MILK.

DETECTION OF SULPHONATED-AZO-DYES.

1. Acidify with Hydrochloric acid.
2. A pink coloration indicates a Sulphonated-azo-dye.

Examine according to Blyth's "Foods: their Composition and Analysis."

DETECTION OF ANNATO, ANILINE ORANGE AND CARAMEL.

Leach's Method.

1. Warm about 150 c.c. of milk in a casserole.
2. Add 5 c.c. Acetic acid.
3. Heat slowly to near the boiling point, stirring constantly.
4. Separate curd from whey by gathering together with stirring rod or by straining.
5. Press the curd free from the adhering liquid.
6. Transfer to a small flask.
7. Add 50 c.c. of Ether and macerate over night, keeping flask tightly stoppered and shaking occasionally.
8. Decant the Ether. Place in an evaporating dish and evaporate. SAVE THE CURD IN ANOTHER DISH IF COLORED.
9. Make the fatty residue alkaline with Sodium Hydrate. Warm and filter while warm.

10. Wash residue from filter with a stream of water. Dry filter. If colored orange, ANNATO is indicated. Confirm by adding a drop of Stannous Chloride Solution. The orange color turns to pink.

11. If curd is colored orange or yellow, ANALINE ORANGE is indicated. Confirm by treating a lump of the fat-free curd in a test-tube with a little conc. Hydrochloric acid. If curd turns pink, the presence of ANALINE ORANGE is assured.

12. If curd be colored a dull brown, CARMEL is to be suspected. Heat a little in a test-tube with strong Hydrochloric acid. The acid will turn deep blue, while the curd remains brown, if CARMEL is present.

NOTE.—In normal milk the solution will turn blue, but the curd remains blue.

Analysis of Butter.

ANALYSIS OF BUTTER.

ESTIMATION OF WATER IN BUTTER.

1. Weigh about 2 grams of butter into a weighed platinum dish, containing a little recently-ignited Asbestos.
2. Heat in water bath until weight is fairly constant.
3. Cool in dessicator.
4. Weigh.
5. Loss in weight is equal to weight of water in weight of butter taken.

NOTE.—Do not keep too long in bath, as the weight will gradually increase after a time.

ESTIMATION OF FAT AND SOLIDS NOT FAT.

1. Dry, and weigh, a fat-free cylinder of blotting paper, as in milk.
2. Place about a gram of butter on the inside of the cylinder.
3. Weigh immediately. Increase in weight is equal to the weight of butter taken.
4. Place in water oven in horizontal position until the melted butter is absorbed by the paper, then, dry further until most of the water has been driven off.
5. Place cylinder in Soxhlet extraction apparatus and extract for $3\frac{1}{2}$ hours with anhydrous Ether.
6. Evaporate Ether extract in weighed flask by aid of suc-

tion. The residue, after being dried to constant weight in water oven, will equal weight of Fat in quantity of milk taken.

7. Remove cylinder from extraction apparatus, dry and weigh. Increase in weight over original weight of cylinder will equal the weight of Solids not Fat + Salt.

ESTIMATION OF ASH IN BUTTER.

1. Carefully ignite residue from water determination until white, being careful not to go above low redness.
2. Cool in dessicator.
3. Weigh.
4. Weight of residue is equal to weight of Ash + weight of Salt in the weight of milk taken.

ESTIMATION OF SALT.

1. Shake up a weighed amount of butter, about 10 grams, in a separatory funnel, with hot water.
2. Titrate water extract with a standard solution of Silver Nitrate, using Potassium Chromate as an indicator.

NOTE.—It is customary to call all butter which contains less than 2 per cent. of salt "Fresh," and all butter containing more than 2 per cent., "Salt."

ESTIMATION OF TOTAL PROTEIDS.

Make a Nitrogen determination as in Milk. Multiply weight of Nitrogen found by 6.25.

ESTIMATION OF CURD.

Subtract the Salt + Ash from the Solids not Fat.

DETECTION OF ADULTERATIONS.

THE ADDITION OF WATER.

Determine by estimating the percentage of water. If this runs over 16 %, the butter has either been adulterated with water, or has been manufactured by a faulty process.

DETECTION OF "PROCESS" BUTTER.

FOAM TEST. *Jour. Amer. Chem. Soc.*, 1900, 22,150.

1. Heat 2 or 3 grams of butter to be tested in a platinum dish. If real butter, it will foam abundantly; if "Process Butter," or "Oleomargarine," it will bump and sputter like hot grease, without foaming.

APPEARANCE TEST. *Jour. Amer. Chem. Soc.*, 1900, 22,327.

1. Heat 100 grams, approximately, of butter at 50° Cent.
2. In real butter the curd will settle, leaving the fat in a clear layer above it. If "Process Butter," the supernatant fat will not be clear.

MICROSCOPIC EXAMINATION, *Jour. Amer. Chem. Soc.*,
1900, 22,150.

1. Place a bit of the sample on a glass slide, cover it and press into a thin film with cover glass. Examine immediately with polarizer at a magnification of from 100 to 200 diameters.

When a Selenite plate is placed between the slide and lower Nicol, butter will give a uniformly colored, blue field; "Process Butter" will give a blue field, mottled with yellow.

THE DETECTION OF "OLEOMARGARINE."

WATERHOUSE TEST, *Jour. Amer. Chem. Soc.*, 1901, 23,200.

1. Half fill a 100 c.c. beaker with sweet milk.

2. Heat nearly to boiling.
3. Add 10 grams of sample butter.
4. Stir with small wooden paddle until fat is melted.
5. Place beaker in cold water and stir until the temperature has fallen below the congealing point of the fat.
6. At this point the fat, if "Oleomargarine," will collect into one lump by using the paddle in the right way. Butter, however, will granulate and be impossible to collect.

THE ADDITION OF FAT OTHER THAN BUTTER FAT.

The detection of this adulteration consists in making several, or all, of the following standard tests, and drawing conclusions from the results as to whether the butter has been adulterated or not.

Blyth gives the following table showing the average values for the standard tests :

	BUTTER.			Marg.	Cocoa.O.
	Mean.	Max.	Min.	Mean.	
Reichert-Woolny	28	33.5	24	.8	7.5-8
Valenta	30°C.	39.C.	20°C.	95.0°C.	
Specific Gravity at $\frac{100^{\circ}}{15^{\circ}}$ C.	.8668	.8685	.8650	.861	.874
Refract. Zeiss at 30° Centigrade .	46	40	43.7	52	41
Koettstorfer Value	227	233	221.5	195	268-246
Insoluble Acids Per Cent.	87.5	90	85.5	95.5	82-90
Soluble Acids Per Cent.	5.0	7.0	4.0	trace	
Iodine Value	34	40	24	55	0-95
Melting Point	32.8°C.	35.0°C.	30.0°C.	26.0°C.	20-28°C.

EXAMINATION OF THE FAT.

DETERMINATION OF VOLATILE FATTY ACIDS.

Reichert-Woolhy Process modified by Löffman and Baum.

1. Melt the butter and keep in a warm, dry place at 60° Cent., for two or three hours until water and curd have entirely deposited.

2. Pour off the supernatant fat and filter through a dry filter, using a hot water funnel. Save.

3. Wash out a distillation flask with water, Alcohol and Ether respectively. Heat for half an hour in oven.

4. Warm a pipette to about 60° Cent., and after mixing the melted fat thoroughly, deliver into the flask, by means of the pipette, exactly 5.75 c.c. of the fat.

5. Add 20 c.c. of Glycerine Soda Solution. (Dissolve 100 grams of NaOH in 100 c.c. of distilled water. Let stand until clear. To 20 c.c. of this solution add 180 c.c. pure concentrated Glycerol).

6. Heat until the mixture becomes clear.

7. Add 135 c.c. distilled water, recently boiled.

N. B.--Add drop by drop at first to prevent foaming.

8. Add 5 c.c. dilute Sulphuric acid.

9. Cool, and add a few pieces of pumice stone. (Prepared by throwing it at a white heat into distilled water and keeping it under water.)

10. Connect flask to a condenser.

11. Distil, carefully at first, so as to produce 110 c.c. of distillate in as near 30 minutes as possible.
12. Filter the 110 c.c. of distillate through a dried filter.
13. Place 100 c.c. of filtrate in a 200 c.c. beaker.
14. Add 1 c.c. of an Alcoholic solution of Phenolphthalein.
15. Titrate with deci-normal NaOH.
16. Multiply result in cubic centimeters by 1.1. This will give the Reichert-Woolny number.

N. B.—Run several blanks.

DETERMINATION OF SOLUBLE AND INSOLUBLE FATTY ACIDS.

Official Method.

SOLUBLE ACIDS.

N. B.—Run at least two blanks

1. Take about 5 grams of sample fat, by means of warmed pipette, run into a clean saponification flask and weigh accurately, having previously obtained the weight of the flask empty. This will give weight of fat taken.
2. Add 50 c.c. of Alcoholic Potash solution, (40 grams of KOH in one liter of 95 % re-distilled Alcohol.) Measure each time with same pipette and let drain thirty seconds.
3. Insert a soft cork and tie down.
4. Place on water bath until the fat is entirely saponified, shaking occasionally.
5. Cool.
6. Remove stopper and wash contents of flask into an Erlenmeyer flask of about 200 c.c. capacity with a little 95 % alcohol.

7. Place on water bath together with blanks until Alcohol has disappeared.

8. Titrate blanks with half-normal Hydrochloric acid, using Phenolphthalein as an indicator.

9. Then run into sample 1 c.c. more of the Hydrochloric acid than is necessary to neutralize the Potash in the blanks.

10. Connect flask with a reflux condenser and place on the water bath until the separated fatty acids form a clear stratum on the surface of the liquid.

11. Cool flask and contents in ice water.

12. After the fatty acids have solidified, pour the liquid contents of flask through a dry filter into a liter flask, care being taken not to break the cake.

13. Add 300 c.c. of water to the flask containing the cake and reinsert the condenser.

14. Heat on steam bath until the cake is thoroughly melted. Agitate at intervals.

15. When separation has again taken place, cool in ice water and filter in same way as before into the flask containing the first filtrate.

16. Repeat this treatment of the cake a third time.

17. Make up the combined filtrates to one liter.

18. Take 100 c.c. of this volume and titrate with deci-normal NaOH.

19. Multiply result by 10. This gives the cubic centimeters of deci-normal solution of alkali. Diminish by 5 to correct for 1 c.c. of half-normal acid added. Multiply final result by .0088. This will give the weight of Butyric acid in amount of fat taken. Calculate to percentage.

INSOLUBLE ACIDS.

20. Allow the flask, containing the cake, together with the filter, to drain and dry for twelve hours.

21. Transfer cake and as much of the fatty acids as can be scraped off the filter to an evaporating dish, previously weighed.

22. The funnel and filter are then placed in an Erlenmeyer flask and the paper thoroughly washed with absolute Alcohol.

23. The flask is then rinsed with the washings just obtained, into the dish. It is then washed ten times with pure Alcohol into the dish.

24. Evaporate to dryness on water bath.

25. Heat in water oven for three hours.

26. Cool in dessicator.

27. Weigh.

28. Weight of residue is equal to the weight of insoluble acids in weight of butter taken. Calculate to percentage.

DETERMINATION OF SAPONIFICATION NUMBER.

Koettstorfer.

N. B.—Conduct at least two blanks.

1. Place from one to two grams of sample fat in a saponification flask.

2. Add 25 c.c. Alcoholic Potash solution. (Use same pipette each time and let drain thirty seconds).

3. Stopper. Heat on water bath until saponification is complete.

4. Cool.

5. Remove cork and add 1 c.c. of Phenolphthalein solution.

6. Titrate blank and sample with half-normal HCl, reading the burette in cubic centimeters.

7. The Koettstorfer number is obtained by subtracting the number of cubic centimeters necessary to neutralize the alkali after saponification from the number necessary to neutralize the

Potash in the blank, multiply this by 28.06 and dividing the product thus obtained by the weight in grams of the fat taken.

VALENTA'S TEST.

Chattaway, Pearmain and Moor.

N. B.—Make several determinations.

1. The butter fat, melted and filtered at room temperature, is further dried by being filtered through a dry filter.
2. Weigh out 2.75 grams of the fat into a stoppered test tube.
3. Add 3 c.c. of 99.5 % Acetic acid.
4. Place in a beaker of water and gradually warm, noting temperature until on shaking the tube a clear solution is obtained. Note temperature at this point carefully.

DETERMINATION OF SPECIFIC GRAVITY.

Proceed the same as in milk, filling the flask, previously standardized with water, with fresh, melted, filtered fat at 100° Cent. Keep in boiling water for 30 minutes and then stopper, the glass stopper having been previously heated to 100° Cent.

Wipe dry and allow to cool. Weigh.

$$\text{Specific Gravity} = \frac{\text{Weight of flask} + \text{Fat.}}{\text{Weight of flask} + \text{Water.}}$$

N. B.—The weight of flask and water should be obtained in the same manner as the weight of flask + Fat.

DETERMINATION OF REFRACTORY INDEX.

Zeiss' Butyro Refractometer.

The directions for this determination are given with each instrument, or can be found described in full in Blyth's "Foods: their Composition and Analysis."

DETERMINATION OF THE IODINE ABSORPTION NUMBER.

OFFICIAL METHOD.

(Bulletin No. 46, Revised Edition. U. S. Department of Agriculture.)

SOLUTIONS.

Iodine Solution—Dissolve 26 grams of pure Iodine in 500 c.c. of 95 % Alcohol. Dissolve 30 grams of Mercuric Chloride in 500 c.c. 95 % Alcohol. Filter the latter solution and mix the two. Let stand twelve hours before using.

Deci-Normal Sodium Thiosulphate Solution—Dissolve 24.6 grams of C. P. Sodium Thiosulphate, freshly pulverized and dried between blotting paper, in distilled water and dilute to one liter at the temperature of the titration.

Starch Paste—Boil one gram of Starch with 200 c.c. of water for ten minutes and cool to room temperature.

Potassium Iodide Solution—Dissolve 150 grams of Potassium Iodide in water and make up to one liter.

Solution of Potassium Di-Chromate—Dissolve 3.874 grams of C.P. Potassium Di-chromate in distilled water and make up to one liter at the temperature of the titration.

STANDARDIZING THE SODIUM THIOSULPHATE SOLUTION.

1. Place 20 c.c. of the Potassium Di-chromate solution in a glass stoppered flask.
2. Add 10 c.c. of Potassium Iodide solution.
3. Add 5 c.c. strong Hydrochloric acid.
4. Run in the Sodium solution from a burette until the yellow color has nearly disappeared.
5. Add a few drops of Starch paste.

6. Add with constant stirring, the Sodium solution, until the blue color has been destroyed.

7. The number of cubic centimeters of the Sodium solution used, multiplied by 5 is the equivalent to one gram of Iodine.

Calculate the value of one cubic centimeter.

DETERMINATION.

1. One gram of the butter fat, as prepared under determination of "Volatile Acids," is weighed into a 300 c.c. stoppered flask.

2. Dissolve completely in 10 c.c. of Chloroform.

3. Add 30 c.c. of Iodine solution.

4. Let stand in a dark place, with occasional shakings, for three hours.

5. Add 100 c.c. distilled water.

6. Add 20 c.c. of Potassium Iodide solution. Wash down any particles of Iodine adhering to the glass with this solution.

7. Run in Sodium solution from burette until the yellow color is nearly gone.

8. Add a few drops of Starch paste.

9. Continue titration with constant shaking until blue color has disappeared for the space of 5 minutes.

10. Read burette.

NOTE--At the time of adding the Iodine solution to the fat, two flasks of the same size should be prepared with all the above reagents as in determination, minus the fat. In every other respect the performance of the blanks should be identical with the determination. These blanks should be run every time the Iodine solution is used.

EXAMPLE BLANK.

30 c.c. of Iodine solution required 46.4 c.c. of Sodium Thio-Sulphate solution. 30 c.c. of Iodine solution required 46.8 c.c. of Sodium solution. Average c.c. Sodium solution equals 46.6 c.c.

Per cent. of Iodine absorbed :

Weight of fat 1.047 grams.
 Quantity of Iodine solution..... 30.0 c.c.
 Sodium solution equivalent of Iodine solution.... 46.6 c.c.
 Sodium solution equivalent to remaining Iodine.. 14.7 c.c.
 Sodium solution equivalent to Iodine absorbed... 31.9 c.c.

$$\frac{31.9 \times 0.124 \times 100}{1.047} \div 7 = 37.75 \text{ Iodine Number.}$$

DETERMINATION OF MELTING POINT.

Wiley's Method.

1. Prepare disks of Fat as follows: allow melted and filtered Fat to fall from a dropping tube on to a smooth piece of ice floating in distilled water. By submerging the ice, the disks can be floated on to a steel spatula previously cooled in ice water.

2. A test tube 30 cm. by 3.5 cm. is nearly filled with a hot mixture of Alcohol and water, (prepared by boiling distilled water and 95% Alcohol separately, to remove any gases which they might contain. The hot water is then poured into the test tube until it is nearly half-full. The hot Alcohol is then poured on top of the water until the tube is nearly full, taking care not to mix the two to any extent.)

3. Place the test tube in a tall beaker filled with ice water until cold.

4. Drop a disk of Fat from the spatula into the Alcohol water. It will float about half-way between the top and bottom.

5. Place a delicate thermometer in the test tube, the bulb being about even with the disk. This thermometer should be gently moved from time to time.

6. Heat water in the beaker gently, stirring constantly.

7. When the disk of fat begins to shrivel, continue the heating with extreme slowness, noting the temperature by the delicate thermometer, constantly.

8. When disk has just taken the form of a sphere, read the thermometer.

NOTE—Repeat the determinations several times.

THE DETECTION OF PRESERVATIVES IN BUTTER.

THE ADDITION OF BORIC ACID.

Detection.

1. Melt 5 grams of sample in dish.
2. Add 5 c.c. of water.
3. Acidify with Hydrochloric acid.
4. Mix while warm.
5. Allow water to separate and test for Borates as in milk.

Estimation. *(Richmond and Harrison's Method.)*

1. Weigh out 25 grams of sample in a beaker.
2. Add 25 c.c. of a solution containing 6 grams of milk sugar and 4 c.c. normal Sulphuric acid to 100 c.c.
3. Place in water oven until fat has just melted. Stir well.
4. Allow aqueous portion to settle well and draw off 20 c.c.
5. Add 1 c.c. of a solution of Phenolphthalein.
6. Bring to a boil.
7. Titrate with half normal Sodium Hydrate until pink just appears.
8. Add 12 c.c. Glycerol and titrate to pink.
9. The difference between these two titrations, less the amount of alkali required by 12 c.c. of Glycerol, multiplied by

.0368, will give the amount of Boric acid in 20 c.c., and this multiplied by 100 = Percentage of Water and divided by 20 will equal the Per cent. of Boric acid.

DETECTION OF OTHER PRESERVATIVES.

See "Preservatives," under Milk.

DETECTION OF COLORING MATTER.

DETECTION OF ANNATTO AND SAFFRON.

Cornwall's Method.

1. Take 5 grams of Fat in a wide tube.
2. Dissolve in 50 c.c. Ether.
3. Shake with 15 c.c. of a very dilute solution KOH.
4. Allow to stand a few hours.
5. Draw off aqueous layer.
6. Evaporate to dryness.
7. Test with Sulphuric acid.

Color changes as follows :

Annatto	=	Blue.....to.....Green.....	Brown.
Saffron	=	Blue.....to	Brown.

ANILINE COLORS.

See Blyth's "Foods : their Composition and Analysis."

Analysis of Cheese.

ANALYSIS OF CHEESE.

DETERMINATION OF WATER.

1. Weigh out about 2 grams of cheese into a weighed platinum dish, containing a little previously ignited Asbestos.
2. Heat in water oven for 12 hours.
3. Cool in dessicator and weigh.
4. Loss of weight is equal to weight of water in the weight of cheese taken. Weight of residue is equal to weight of Total Solids.

DETERMINATION OF FAT.

(*U. S. Dept. of Agr., Div. of Chem. Bul. 36 revised, p. 36.*)

1. Weigh out 2 grams of finely divided cheese into a Soxhlet extraction tube, on the bottom of which is a layer of Asbestos covered with a mixture of anhydrous Cupric Sulphate and pure dry sand to the depth of 5 cm.
2. Extract with anhydrous Ether for five hours.
3. Remove cheese from tube and grind in a mortar to a fine powder with a little pure sand.
4. Replace in extraction tube, washing out mortar with Ether into the tube.
5. Continue extraction for ten hours.
6. Evaporate Ether extract in weighed flask. Dry residue in water bath to as constant a weight as possible and weigh.
7. Weight of residue is equal to the weight of Fat in the weight of cheese taken.

DETERMINATION OF PROTEIDS.

Obtain by subtracting the weight of all the other constituents from 100. This method is preferable to determining the Nitrogen and then multiplying by 6.25 on account of varying percentage of Nitrogen in cheese proteids.

DETERMINATION OF NITROGEN.

Determine, as in milk, by the Kjeldahl process, using 2 grams of cheese.

SEPARATION OF FAT FOR EXAMINATION.

(U. S. Dept. of Agr., Div. of Chem. Bul. 31.)

1. Cut 300 grams of cheese into fragments the size of a pea.
2. Treat in a flask, with 700 c.c. of KOH solution (50 grams per liter) at 20° Cent., shaking vigorously at intervals.
3. Collect the lumps of fat into as large a mass as possible by shaking to and fro.
4. Add cold water until fat is driven up the neck of the flask and can be removed by means of a spoon.
5. Wash with cold water to free from Potassium Hydrate.
6. Examine fat as in butter.

DETECTION OF ADULTERATIONS.

Besides the addition of other fats than butter fat and the adding of an excess of water, there are few adulterations to be looked for. Preservatives (?) may be detected as in milk and butter, and coloring matters in the same way. The rind of the cheese should be examined for mineral poisons, especially Lead and Arsenic compounds.

Appendix I.

United States Department of Agriculture,

OFFICE OF THE SECRETARY—CIRCULAR NO. 10.

Standards of Purity for Food Products.

FOOD DEFINITIONS AND STANDARDS.

I. ANIMAL PRODUCTS.

B. MILK AND ITS PRODUCTS.

a. MILKS.

Definition.

Milk (*whole milk*) is the lacteal secretion obtained by the complete milking of one or more healthy cows, properly fed and kept, excluding that obtained within fifteen days before and five days after calving.

Standard.

Standard milk is milk containing not less than twelve (12) per cent. of total solids and not less than eight and one-half (8.5) per cent. of solids not fat, nor less than three and one-quarter (3.25) per cent. of milk fat.

Definitions.

2. *Blended Milk* is milk modified in its composition so as to have a definite and stated percentage of one or more of its constituents.

3. *Skim milk* is milk from which a part or all of the cream has been removed.

Standard.

Standard Skim Milk is skim milk containing not less than nine and one-quarter (9.25) per cent. of milk solids.

4. *Buttermilk* is the product that remains when butter is removed from milk or cream in the process of churning.

5. *Pasteurized milk* is standard milk that has been heated below boiling but sufficiently to kill most of the active organisms present and immediately cooled to fifty degrees (50°) Fahr. or lower to retard the development of their spores.

6. *Sterilized milk* is standard milk that has been heated at the temperature of boiling water or higher for a length of time sufficient to kill all organisms present.

7. *Condensed milk* is milk from which a considerable portion of water has been evaporated.

8. *Sweetened condensed milk* is milk from which a considerable portion of water has been evaporated and to which sugar (sucrose) has been added.

Standard.

Standard condensed milk and *standard sweetened condensed milk* are condensed milk and sweetened condensed milk, respectively, containing not less than twenty-eight (28) per cent. of milk solids, of which not less than one-fourth is milk fat.

9. *Condensed skim milk* is skim milk from which a considerable portion of water has been evaporated.

b. MILK FAT OR BUTTER FAT.

Definition.

1. *Milk fat* or *butter fat* is the fat of milk.

Standard.

Standard milk fat or *butter fat* has a Reichert-Meissl number not less than twenty-four (24) and a specific gravity not less than 0.005 (40° C. / 40° C.).

c. CREAM.

Definition.

1. *Cream* is that portion of milk, rich in butter fat, which rises to the surface of milk on standing, or is separated from it by centrifugal force.

Standard.

Standard cream is cream containing not less than eighteen (18) per cent. of milk fat.

2. *Evaporated cream* is cream from which a considerable portion of water has been evaporated.

d. BUTTER.

Definition.

1. *Butter* is the product obtained by gathering in any manner the fat of fresh or ripened milk or cream into a mass, which also contains a small portion of the other milk constituents, with or without salt. By acts of Congress approved August 2d, 1886, and May 9th, 1902, butter may also contain additional coloring matter.

Standard.

Standard butter is butter containing not less than eighty-two and five-tenths (82.5) per cent. of butter fat.

Definition.

2. *Renovated or process butter* is the product obtained by melting butter and reworking, without the addition or use of chemicals or any substances except milk, cream, or salt.

Standard.

Standard renovated or process butter is renovated or process butter containing not more than sixteen (16) per cent. of water and at least eighty-two and five-tenths (82.5) per cent. of butter fat.

L. of N.

e. CHEESE.

Definitions.

1. *Cheese* is the solid and ripened product obtained by coagulating the casein of milk by means of rennet or acids, with or without the addition of ripening ferments and seasoning. By act of Congress, approved June 6, 1896, cheese may also contain additional coloring matter.

2. *Whole milk or full cream cheese* is made from milk from which no portion of the fat has been removed.

3. *Skim-cream cheese* is cheese made from milk from which any portion of the fat has been removed.

4. *Cream cheese* is cheese made from milk and cream, or milk containing not less than six (six) per cent. of fat.

Standard.

Standard whole-milk or full-cream cheese is whole-milk or full-cream cheese containing in the water-free substance, not less than fifty (50) per cent. of butter fat.

Appendix II.

**The Babcock Method of Determining the
Percentage of Fat in Milk.**

The Babcock Method of Determining the Percentage of Fat in Milk.

Of all the testing machines and instruments on the market for the purpose of determining approximately the percentage of fat in milk, the only ones which have any value whatsoever are those depending on the solution of the casein by means of concentrated sulphuric acid and the final separation of the fat by centrifugal force.

Of the machines operating on this principle, perhaps the Babcock tester is the best known and most widely used. The outfit consists of a set of bottles, in which the separation is made, a centrifugal machine, a 17.6 c.c. pipette and a 17.5 c.c. graduate.

The bottles are of about 35 c.c. capacity, with long, thin, graduated necks, the graduations reading directly in percentage of fat when 17.6 c.c. or 18 grams of milk are used. The test is conducted as follows :

1. Add 17.6 c.c. of milk to one of the bottles by means of the graduated pipette.
2. Add carefully, 17.5 c.c. of concentrated sulphuric acid, by means of the graduated cylinder, adding the acid little by little and rotating the bottle so as to give a rotary motion to the liquid within. When the acid has been completely added and the liquid thoroughly mixed, it should be of a uniform dark brown or black color.
3. Having filled an even number of bottles with milk and acid, place them while still hot in the centrifugal machine, being

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